ine or flunarizine to further reduce the risk of unwanted excitatory effects.

In sum, it seems that future rTMS protocols will most likely need distinctly *fewer* stimuli and *less* intensity—and hence less energy—to produce even longer and much clearer inhibitory and facilitatory aftereffects, but there is still much work to be done. Clearly, it will need to be investigated whether combining rTMS with other methods will in fact improve its therapeutic efficacy. Also, for areas outside the primary motor cortex, other methods for the quantification of TBS effects, beyond measuring motor evoked potentials, will be needed. rTMS in combination with functional fMRI may be one solution (Bestmann et al., 2004).

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Progress in Realizing the Promise of Microarrays in Systems Neurobiology

The power of microarrays in neuroscience has been challenged by the cellular heterogeneity and complexity of the central nervous system. In this issue of *Neuron*, Arlotta, Molyneaux, and colleagues have developed a technique combining retrograde labeling, flow cytometry, and microarrays to purify and molecularly characterize a specific population of neurons from the brain, focusing here on cortical projection neurons. We discuss these findings and the implications of this development for both systems and molecular neuroscience.

Advances in technology clearly drive discovery in science, but often there is a lag between development of the technology and optimization of its implementation. This year marks the tenth anniversary of the birth of the cDNA microarray (Schena et al., 1995), which was closely followed by the development of the oligonucleotide arrays for gene expression analysis (Lockhart et al., 1996). Thus, it is an opportune time to reflect on what microarrays have contributed to our understanding of the nervous system.

Initially, the greatest successes with microarrays were in applications with homogeneous cell culture systems, such as yeast or T cells, and they were rapidly applied to the study of cancer and metabolic regulation (DeRisi et al., 1996; Lockhart et al., 1996; Shalon et al., 1996). Many neuroscientists were initially skeptical of the application of these powerful methods to a complex tissue such as the brain. Nonetheless, many studies proceeded to characterize the gene expression profile of a variety of CNS diseases and of fundamental nervous system properties such as regional specificity, species differences, or development and aging (reviewed in Geschwind, 2003; Mirnics and Pevsner, 2004; Preuss et al., 2004). While these studies invariably identified useful lists of differentially regulated genes, they all dealt with the limitations imposed by cellular heterogeneity in a post hoc manner, if at all (Geschwind, 2000). The pioneering use of laser capture microdissection (LCM) to study morphologically and immunochemically identifiable dorsal root ganglion neurons provided a notable exception (Luo et al., 1999).

There are two major difficulties posed by cellular heterogeneity in microarray studies. First, and most importantly, cellular heterogeneity restricts the detection of gene expression changes. For example, if a gene is expressed in a rare cell type, even massive changes in its expression may not be detected, because it represents only a small fraction of the RNAs measured. Also, biologically important changes in gene expression in one cell type may be masked by compensatory changes in another cell type. Second, even when changes in gene expression are detected in a heterogeneous tissue, we cannot determine what biological changes are responsible for the result. Possible causes of the observed change in gene expression could include change in the proportion of cell types (such as a loss of neurons or gliosis), change in expression in all cells, change in expression in a fraction of cells, invasion of a new cell type (such as inflammatory cells), or combinations of all of these.

It is in this context that work reported in this issue of *Neuron* by Arlotta, Molyneaux, and colleagues presents an elegant solution to the problem of CNS heterogeneity by applying a combination of classic anatomical techniques and flow cytometry to isolate a specific subpopulation of neurons from the cerebral cortex for microarray analysis: developing corticospinal motor neurons (CSMN) (Arlotta et al., 2005). It is important to realize that, rather

than depending on molecular markers, such as antigens, the strategy employed by these authors studies gene expression in the context of an integrated neural system that is central to motor behavior, relying on the in vivo functional connectivity of the specific neurons studied. This tour-de-force study provides a glimpse of the direction in which the field is headed: away from bulk characterization of gene expression in heterogeneous cerebral cortical tissue and toward elegant strategies that will allow us to bridge the gap between systems-level neurobiology and the molecular mechanisms that must underlie a system's operation. This combination of techniques, and others likely to follow, permits the study of specific neural systems at a level of molecular genetic analysis previously unattainable.

In the current study, Arlotta, Molyneaux, and colleagues fluorescently labeled specific populations of neurons in the developing mouse brain by retrograde labeling based on their axonal projection targets. Using ultrasound targeting, the authors injected green fluorescent microspheres into three specific anatomical targets containing axons of CSMN, callosal projection neurons (CPN), or corticotectum projection neurons (CTPN) in developing mice. The axons absorbed the microspheres and transported them retrogradely from the spinal cord or pons to label their cell bodies in layer V of the cortex. The authors then coupled this labeling to dissection, enzymatic dissociation, and florescence-activated flow sorting to purify the labeled neurons, using techniques they had previously developed to examine growth factor dependence of these cells (Catapano et al., 2001). However, in this study, they instead combined their purification with gene expression profiling on Affymetrix mouse genome arrays, allowing them to observe the gene expression of these different populations of cells at four different developmental stages: E18, P3, P6, and P14. Remarkably, they were able to identify genes expressed specifically in each of these anatomically intermixed populations of neurons, something that would not have been possible using whole cortex. They were also able to identify genes specific to different stages of development in all projection neurons, suggesting differential gene expression for neuronal processes of pathfinding, axon elongation, synapse formation, and maturation.

The authors confirmed their results in independent samples with extensive in situ hybridization and immunohistochemistry. This anatomical work confirmed that 14 of the candidates identified as CSMN specific by microarray were indeed expressed in these cells in vivo by morphological criteria and double labeling with retrograde markers. But are these genes functionally important to the specification of CSMN fate in vivo? In a clear demonstration of the effectiveness of their approach, they characterized the CNS phenotype of a knockout mouse of *Ctip2*, a gene discovered in their screen to be expressed specifically in the cortex in neurons projecting subcerebrally, including CSMN. These animals have dramatic defects in the development of their descending cortical tracts, including a lack of fasciculation in the internal capsule, misguided and dysmorphic growth cones, and premature termination of the corticospinal tract, such that no axons extend past the pons. Interestingly, the heterozygote animals have more subtle defects in CSMN fasciculation, suggesting that haploinsufficiency of *Ctip2* has phenotypic consequences. The authors further pursue the function of *Ctip2* by showing that it plays a role in pruning of what are normally mostly transient, exuberant connections: *Ctip2* mutants contain a large number of aberrant spinal cord projections far past the period when these connections are normally withdrawn. Thus, Arlotta, Molyneaux, and colleagues extend their genomic investigation of motor neuron development far beyond the phase of marker identification and verification, to a demonstration of functional relevance, providing a solid proof of principle.

While there is no question that the data presented clearly demonstrate the effectiveness of this approach, there are at least three, mostly technical, issues that should be addressed so as to continue to mature the development of this and similar techniques for widespread systems-level neuroscience application. The first issue is that changes in gene expression are dynamic, and certainly some of the gene expression measured in these cells must be in response to the trauma of dissociation and sorting. Indeed, in cell culture experiments with postnatal CPN, 80%–90% of these cells would die within 2 days in vitro (Catapano et al., 2001). This factor is surmountable, since most reactive changes are not cell type specific. Here, the authors carefully controlled for this confound by comparing CSMN to similarly dissociated CPN and collected RNA immediately after sorting. Follow-up studies, such as in situ hybridization, clearly demonstrate the effectiveness of this design and the relevance of the gene expression changes identified to true in vivo biology. The second issue, although clearly not a concern for the current, developmental study, is the need to determine the extent to which techniques based on cell sorting can be applied effectively to adult CNS tissue, which is significantly more difficult to dissociate than developing brain. Adapting this technique to the adult brain would allow examination of gene expression profiles of specific subpopulations of fully mature adult neurons or examination of changes in circuitry as a consequence of aging, disease, and degeneration. Finally, there is a more general challenge to those using microarrays: the scale of array data interpretation has always lagged behind the scale of data generation. Results of array studies provide an entire simultaneous profile of gene expression, on the scale of hundreds to thousands of genes, yet interpretation is typically approached gene by gene. Arlotta, Molyneaux, and colleagues approach this problem by placing genes into five general functional classes reducing the high dimensionality of the data and placing them in appropriate neurobiological context. Yet in the future, more sophisticated analytic tools will enable an even more holistic understanding of gene expression. Already, approaches for understanding expression profiles as a whole are beginning to become available (Bergmann et al., 2004), and for systems molecular neuroscience to come of age, we need to consider entire patterns, or networks of gene expression, in addition to identifying a few key candidates.

This study opens doors to many novel approaches for systems neurobiology: identifying genes specifically expressed in subpopulations of neurons, based on their position and projections in the CNS, also identifies promoters that could be exploited to express proteins spe-

cifically in those neurons. Coupled to transgenic strategies, this would allow for genetic tagging, manipulation, or ablation of specific neuronal subpopulations, permitting studies concerning the function of those cells that are more precise than ever before possible. Furthermore, a catalog of the healthy gene expression profiles of all major neuronal subtypes, based on their in vivo connectivity, is now attainable, providing a baseline for understanding normal neuronal functioning and a comparator for diseased states. And already some are pioneering the gene expression profiling of single cells, which will allow a more complete understanding of the diversity of individual neurons within populations (Tietjen et al., 2003).

It is also important to note that CSMN are of particular interest in health and disease because they are essential for voluntary control of motor systems and are involved in amyotrophic lateral sclerosis and other degenerative diseases involving motor systems. This particular study, by providing some insight into their early gene expression during development and maturation, may provide targets for genetic manipulation to encourage neuronal progenitors to assume a CSMN fate, as well as yield information on which pathways are essential for their normal integration into CNS circuitry. So, after nearly 10 years, through the work of these authors and others, microarrays are beginning to realize their promise of becoming a tool for systems molecular neuroscience, enabling understanding of the unique gene expression profile of specific subsets of neurons with known roles in brain circuits and behavior.

We dedicate this article to the memory of our colleague Zheng Luo.

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The Molecular Machinery of Resurgent Sodium Current Revealed

Some TTX-sensitive sodium channels open transiently during recovery from inactivation, generating a "resurgent" sodium current that flows immediately following action potentials. In this issue of *Neuron*, Grieco and colleagues present evidence that resurgent sodium current results from a novel form of inactivation in which the cytoplasmic tail of the β4 subunit acts as a classic open-channel blocker.

Few concepts in neurophysiology seem as basic as the refractory period: sodium channels inactivate during the action potential, and the neuron is inexcitable until they recover. Although the concept of the refractory period may be straightforward for action potentials in axons (where it was originally described experimentally and later explained mechanistically by Hodgkin and Huxley), the cell bodies of some neurons behave very differently: stimulation of a single spike, even by a brief, just-suprathreshold stimulus, can be followed by firing of multiple spikes in an all-or-none manner—a kind of antirefractory behavior. Cerebellar Purkinje neurons, which behave in this way (Callaway and Ross, 1997; Raman and Bean, 1997), turn out to possess TTX-sensitive sodium currents with unusual gating behavior: upon depolarization, currents activate and inactivate in a normal fashion, but when the cell is repolarized to voltages near −50 or −60 mV (where normal sodium channels would remain closed as they recover from inactivation), a surge of sodium current flows transiently (Raman and Bean, 1997). This "resurgent" current is correlated with unusually rapid recovery from inactivation (Raman et al., 1997). Thus, even as the surge of current provides a depolarizing influence, channels are being quickly reprimed, and both events promote the firing of a second action potential.

The molecular basis of resurgent sodium current has been a puzzle. In Purkinje neurons, it appears to be carried mainly by $Na_v1.6$ pore-forming α subunits, one of the three types of sodium channels with widespread distribution in the nervous system, since it is reduced to 10%–20% of wild-type levels in $Na_v1.6$ null mice (Raman et al., 1997). However, the fact that some resurgent current remains in $Na_v1.6$ null animals—even more evident in subthalamic nucleus neurons (Do and Bean,